AMENDMENTS TO THE SPECIFICATION

The amendments to the specified portions of the specification will replace all previous versions of that portion of the specification. No new matter is introduced by way of the amendments to the specification.

Page and paragraph references cited hereinbelow are taken from United States Patent publication 20060217317.

Amended Paragraph [0019] on Page 2

Please replace paragraph [0019] on page 2 with the following replacement paragraph. Amended paragraph [0019] contains SEQ ID references to the peptide sequences listed in FIG. 2.

[0019] FIG. 2 depicts a graph of hK2 mediated hydrolysis of peptide substrates identified through the screening of a random peptide library [Y'GKARAF-Dap-FK' (SEQ ID NO:36), Y'GKAKPR-Dap-FK' (SEQ ID NO:37), Y'GKAFRR-Dap-FK' (SEQ ID NO:38), Y'GKAMRQ-Dap-FK' (SEQ ID NO:39) and Y'GSKGHFKL-Dap-F-K' (SEQ ID NO:40)]. Substrates (250 μM) were incubated with 8 μg/ml hK2 in PBS buffer. Hydrolysis of peptide results in increased fluorescence, as measured using a 96 well fluorometer. Note that the double arginine substrate (Y'GKAFRR-Dap-FK', SEQ ID NO:38) by far exceeded all other substrates. A substrate without arginine (Y'GSKGHFKL-Dap-F-K', SEQ ID NO:40) did not show any hydrolysis. To determine 100% digestion, trypsin was added (50 mg/ml) and samples were incubated to 37° C. for 30 min and the fluorescence was determined. The percentage digested is determined by dividing fluorescence units of sample by fluorescence units of control fully digested peptide.

Amended Paragraph [0020] on Page 2

Please replace paragraph [0020] on page 2 with the following replacement paragraph. Amended paragraph [0020] contains a SEQ ID reference for Ac-GKAFRR-L12ADT.

[0020] FIG. 3 depicts the chemical structure of the hK2 prodrug, Ac-GKAFRR-L12ADT (SEQ ID NO:41). HK2 cleavage sites are indicated. The ratio of RL-12ADT:L-12ADT generated by hK2 digestion was 1:1.8.

Amended Paragraph [0021] on Page 2

Please replace paragraph [0021] on page 2 with the following replacement paragraph. Amended paragraph [0021] contains a SEQ ID reference for Ac-GKAFRR-L12ADT.

[0021] FIG. 4 depicts HPLC analysis of the hydrolysis of the hK2 prodrug Ac-GKAFRR-L12ADT (SEQ ID NO:41) by hK2 (4 μMg/ml) incubated in 50 mM Tris, 0.1 M NaCl, pH 7.8 at room temperature for 24 hours. The mass of each peak was confirmed by MALDI-TOF mass spectrometric analysis.

Amended Paragraph [0022] on Page 2

Please replace paragraph [0022] on page 2 with the following replacement paragraph. Amended paragraph [0022] contains a SEQ ID reference for Ac-GKAFRR-L12ADT.

[0022] FIG. 5 depicts the stability of Ac-GKAFRR-L12ADT (SEQ ID NO:41) in human plasma after a 24 hour incubation, as determined by HPLC analysis. Ac-GKAFRR-L12ADT (SEQ ID NO:41) was incubated in 50% human plasma for 24 hours at room temperature. Peak 1 represents an unidentified plasma contaminant that was also present in control plasma. Peaks 2 and 3 both represent Ac-GKAFRR-L12ADT (SEQ ID NO:41) as confirmed by MALDI-TOF mass spectrometric analysis.

Amended Paragraph [0024] on Page 2

Please replace paragraph [0024] on page 2 with the following replacement paragraph. Amended paragraph [0024] contains SEQ ID references for the peptide sequences listed in FIG. 7.

[0024] FIG. 7 depicts hydrolysis of hK2 peptide substrates [Y'GKAFRRLGK' (SEQ ID NO:21) and Y'GKAFRR-Dap-GK' (SEQ ID NO:42)] in plasma. Arginine containing lead hK2 substrates (500 mM) were incubated in 50% mouse or human plasma. Generation of fluorescence indicated

that the fluorescence-quenched peptides are unstable in plasma. Hydrolysis of the substrates was confirmed by HPLC. Comparison of mouse and human plasma for the same substrate suggest higher proteolytic activity in mouse plasma compared to human plasma.

Amended Paragraph [0025] on Page 2

Please replace paragraph [0025] on page 2 with the following replacement paragraph. Amended paragraph [0025] contains SEQ ID references for acGKAFRRL-12ADT and acGKAFRRLG.

[0025] FIG. 8 depicts HK2 mediated hydrolysis of various peptide and prodrug substrates (125 mM each). Fluorescent substrates were analyzed by means of a fluorescence plate-reader (ex=355 nm, em=460 nm). acGKAFRRL-12ADT (SEQ ID NO:41) and acGKAFRRLG (SEQ ID NO:43) were analyzed by HPLC and quantified by HLPC integration.

Amended Paragraph [0026] on Page 2

Please replace paragraph [0026] on page 2 with the following replacement paragraph. Amended paragraph [0026] contains a SEQ ID reference for GKAFRRL-12ADT. Amended paragraph [0026] also contains a correction of a minor typographical error.

[0026] FIG. 9 depicts pharamacokinetic analysis of GKAFRR-L12ADT (SEQ ID NO:44) following single intravenous injection of 3.67 μmole/kg. Mice were treated in groups of 3 and then sacrificed at indicated time points. Data represent average ± standard error of plasma concentrations as determined by LC-MS analysis.

Amended Paragraph [0030] on Page 3

Please replace paragraph [0030] on page 3 with the following replacement paragraph. Amended paragraph [0030] contains the SEQ ID reference for G-K-A-X_1-X_2-X_3 (set forth subsequently in paragraph [0057].

[0030] Preferably, the peptide sequences of the invention comprise the sequence G-K-A-X_1-X_2-X_3 (SEQ ID NO:17), wherein at least one of X_1, X_2, and X_3 is an arginine residue and wherein the amino acid residues at the other two positions of X_1, X_2, and X_3 are any amino acid residue.

hK2 may cleave the peptide after either X_1, X_2, or X_3, and in the most preferred embodiments, hK2 cleaves the peptide after an arginine residue. Specific preferred sequences (including cleavage sites) are shown in FIG. 1 (SEQ ID NOS:1-14). Further preferred sequences in include the sequences shown in FIG. 1, with an additional leucine residue after the X_3 position (SEQ ID NOS:22-35). In a particularly preferred embodiment, the peptides of the invention comprise the amino acid sequence of SEQ ID NO:9.

Amended paragraph [0079] on Page 8

Please replace paragraph [0079] on page 8 with the following replacement paragraph. The SEQ ID NO previously set forth for GKAFRRL in this paragraph is incorrect. SEQ ID NO:30, as previously set forth in paragraph [0019], should be assigned to the sequence GKAFRRL.

[0079] The peptide sequence GKAFRRL (SEQ ID NO:30~~-18~~) was synthesized on a Rainin PS3 automated peptide synthesizer on Fmoc-Leu-Wang resin (100 μmoles scale). The same protecting groups were used as during the combinatorial synthesis, except for the lysine, which was orthogonally protected with the ivDde group (Fmoc-Lys(ivDde)-OH, Novobiochem). After deprotection of the N-terminal glycine, the amine was acetylated with acetic anhydride and NMM.

Amended Paragraph [0080] on Page 8

Please replace paragraph [0080] on page 8 with the following replacement paragraph. Amended paragraph [0080] contains a SEQ ID reference for acGKAFRRL-12ADT. Amended paragraph [0080] also contains a correction of a minor punctuation error.

[0080] Deprotection of the acid-labile protecting groups and purification was performed as outlined above. Boc-12 ADT was synthesized as previously described (Jakobsen, C. M. et al. (2001) J. Med. Chem. 44:4696-4703). TFA treatment, followed by semi-prep HPLC and lyophilisation afforded the amine containing 12ADT. The protected peptide (ac-GK(ivDde)AFRRL) was coupled to 12ADT after Hobt/DIC activation. After completion of the reaction, the ivDde group was removed by adding hydrazine to the reaction mixture (2% final,

30 min). Semi-preparative HPLC yielded acGKAFRRL-12ADT (SEQ ID NO:41), typically in 60-70% yield. Product was confirmed by MALDI-TOF analysis.

Amended Paragraph [0081] on Page 8

Please replace paragraph [0081] on page 8 with the following replacement paragraph. Amended paragraph [0081] contains a SEQ ID reference for acGKAFRRL-12ADT.

[0081] Calibration standards consisted of ac-GKAFRR-L12ADT (SEQ ID NO:41) prodrug, RL12ADT or L12ADT spiked into mouse plasma and plasma samples from treated mice were analyzed by liquid chromatography coupled to a quadrupolequadripole mass spectrometer (LC/MS/MS) [PESciex API 3000]. A multistep gradient elution HPLC method was used to separate the ac-GKAFRR-L12ADT (SEQ ID NO:41) prodrug from the free RL12ADT and L12ADT with eluent A=2 mM ammonium acetate with 0.1% formic acid 1% acetic acid in deionized water and eluent B=90% acetonitrile/1% acetic acid/0% deionized water. Samples were eluted through a Zorbax SB-C18 Rapid Resolution column (2/1x30 mm, 3.5 μm) at a flow rate of 0.3 ml/min and gradient of 100% A to 100% B over 12 minutes. Calibration was done using extracted standards of ac-GKAFRR-L12ADT (SEQ ID NO:41) added to and then extracted from mouse plasma in a range of 0.001-10 μM, and linear regression analysis was used to generate best-fit lines, from which peak areas of samples were converted to concentration of prodrug. Peak areas of RL12ADT and L12ADT were below limit of detection at all time points and, therefore, calibration was not performed. Single-dose pharmacokinetics were assessed by noncompartmental analysis (Gibaldi, M. and Perroer, D. Pharmacokinetics, 2nd edition, p. 407-409. New York, 1982) The area under the curve from time zero to infinity ($AUC_{0-\infty}$) was calculated with the linear trapezoidal method (Gibaldi, M. and Perroer, D. Pharmacokinetics, 2nd edition, p. 407-409. New York, 1982)). The terminal half-life (T1/2) was determined from the terminal slope (ke) on a log-linear plot of concentration versus time.

Amended Paragraph [0082] on Page 8

Please replace paragraph [0082] on page 8 with the following replacement paragraph. Amended paragraph [0082] contains a SEQ ID reference for acGKAFRRL-12ADT. Amended paragraph [0082] also contains a correction of a minor punctuation error.

[0082] To determine in vivo toxicity of ac-GKAFRR-L12ADT (SEQ ID NO:41), Balb-C mice (Harlan) received a single intravenous injection of an increasing dose of prodrug. Mice were monitored for toxicity hourly for twelve hours and then daily x one week. Separate groups of three mice each received increasing doses of ac-GKAFRRL12ADT (SEQ ID NO:41). Dose escalation was stopped at the dose level that resulted in death of all mice after 24 hours (i.e. LD_{100}). All animals receiving doses less than LD_{100} were alive and well up to 1 week after receiving a single dose. All procedures were performed according to protocols approved by the Johns Hopkins Animal Care and Use Committee.

Amended Paragraph [0089] on Page 9

Please replace paragraph [0089] on page 9 with the following replacement paragraph. Amended paragraph [0089] contains a SEQ ID reference for Y'GSKGHFKL-Dap-K', Y'GSKGPFKL-Dap-K', and Y'GSKGHFHL-Dap-K'.

[0089] Seven out of fourteen peptides contained one or more arginine residues. The peptides lacking any arginine did not show a specific amino acid preference. In order to confirm that the selected sequences represented true hK2 substrates and not false positives, the majority of the peptides were re-synthesized, cleaved from the resin and tested for hydrolysis by hK2 in solution. After re-synthesis, the soluble non-arginine containing peptides were not hydrolyzed by hK2, confirming the suspicion that the arginine-free sequences were not hK2 substrates but false positives probably generated by a combination of events. An additional attempt to identify arginine-free hK2 substrates was made by replacing arginine with lysine in the P1 position of known hK2 substrates. The P1-arginine in the SgI/II sequence GSKGHFRL (SEQ ID NO:19) was substituted for lysine and tested in solution as a fluorescence-quenched peptide as well as derivatives of this sequence [{Y'GSKGHFKL-Dap-K' (SEQ ID NO:45) and Y'GSKGPFKL-Dap-K' (SEQ ID NO:46)]. The arginine-free sequence GSKGHFHL (SEQ ID NO:20), identified as a substrate in the SPOT analysis was also synthesized for testing in solution [{Y'GSKGHFHL-Dap-K' (SEQ ID NO:47)]. Once again, none of these three arginine free peptides were digested by hK2, even after prolonged incubation. These results further support results from earlier studies using small peptide substrates and phage display and reaffirm that hK2 has a strict requirement for arginine in the P1 position of peptide substrates.

Amended Paragraph [0090] on Page 9

Please replace paragraph [0090] on page 9 with the following replacement paragraph. Amended paragraph [0090] contains a SEQ ID reference for Y'GKAFRR-Dap-F-K'. Amended paragraph 90 also contains a correction of minor typographical errors.

[0090] The combinatorial screen identified seven arginine-containing peptides. Four of these were re-synthesized (X_1-X_2-X_3=RAF, KPR, FRR and MRQ respectively). Three other lead sequences were not re-synthesized (X_1-X_2-X_3=IQR, FRK and VRQ respectively). All four arginine-containing sequences that were re-synthesized reproduced fluorescence when these peptides were digested on-bead with hK2. For a more quantitative analysis, the fluorescence-quenched peptides were cleaved off the resin and purified by HPLC. The rate of hydrolysis was quantified by measuring increase in fluorescence (Figure). The best substrate proved to be the sequence with arginine at P1 and P2 [(i.e. Y'GKAFRR-Dap-F-K' (SEQ ID NO:38)]. In less than five minutes, more than 50% of the peptide was digested (500 μM peptide, 4 μg/ml hK2). For the other peptides, digestion of the same amount of peptide took 19-29 minutes. Maximum digestion never exceeded more than 70-75%, a value that was reached with Y'GKAFRR-Dap-F-K' (SEQ ID NO:38) in less than 15 minutes. In subsequent studies, hydrolysis rates using the Y'GKAFRR-Dap-F-K' (SEQ ID NO:38) peptide were analyzed by Lineweaver-Burke reciprocal plots. The Michaelis-Menten constant (K_m) was determined at 26.5 μM, the k_{cat} at 1.09 sec^{-1} and the k_{cat}/K_m ratio was 41,132 sec^{-1} M^{-1}. These results compare favorably[[e]] to those previously reported for the Pro-Phe-Arg-AMC substrate used to assay hK2 activity (K_m 40 μM; k_{cat} 0.92 sec^{-1}; k_{cat}/K_m 22,916 sec^{-1} M^{-1}) and were superior to the GKAFR-AMC substrate we generated based on results of SPOT analysis (K_m 146 μM; k_{cat} 0.13 sec^{-1}; k_{cat}/K_m 895 sec^{-1} M^{-1}).

Amended Paragraph [0091] on Page 10

Please replace paragraph [0091] on page 10 with the following replacement paragraph. Amended paragraph [0091] contains a SEQ ID reference for Y'GKAFRR-Dap-GK' and Y'GKAFRRLGK".

[0091] Arginine-containing peptides are potential substrates for the wide variety of other trypsin-like proteases that are present in the blood and may have residual activity in the blood. The

plasma stability of an hK2 peptide substrate may therefore be limited and this would have significant consequences related to the development of an hK2 activated prodrug. Therefore, two fluorescence quenched hK2 peptide substrates peptides were incubated in 50% mouse or human plasma (diluted in PBS buffer) to determine stability using a plate-reader. As observed with arginine containing peptides from the SPOT analysis above, none of the arginine-containing, fluorescence quenched hK2 peptide substrates were stable in human or mouse plasma. Fluorescence-quenched Y'GKAFRR-Dap-GK' (SEQ ID NO:42) and Y'GKAFRRLGK' (SEQ ID NO:21) (500 μM each) were hydrolyzed when incubated in 50% mouse or human plasma (FIG. 7). Mouse plasma degraded the peptides faster than human plasma (FIG. 7). The Leucine containing peptide was less stable than the Dap containing peptide in both plasma types. HPLC analysis of the peptides after 3 hours of incubation confirmed that the fluorescence generated during this plate reader assay corresponded with proteolysis; almost no parent peptide remained after 3 hours in mouse plasma (~%). In human plasma, more peptide remained after 3 hours (~25%). Several degradation products were generated, probably caused by the action of several exo- and endoproteolytic activities. Overnight incubation in human plasma resulted in complete degradation of both peptides. The proteolytic activity from plasma was never affected by repeated freeze-thaw cycles or by storage of the mouse plasma alone at room temperature for several days.

Amended Paragraph [0093] on Page 10

Please replace paragraph [0093] on page 10 with the following replacement paragraph. Amended paragraph [0093] contains a SEQ ID reference for ac-GKAFRR-L12ADT. Additionally, the SEQ ID NO for ac-GKAFRRLG previously set forth in this paragraph is incorrect. SEQ ID NO:22 that was previously assigned to ac-GKAFRRLG is properly assigned to the sequence GKARAFL, as set forth in paragraph [0018]. Sequence ac-GKAFRRLG is now assigned SEQ ID NO:43.

[0093] To synthesize an hK2 activated prodrug, the sequence C-terminal of the cleavage site (Dap-F-K') must be replaced by L12ADT and the N-terminal Y' replaced by an amino terminal acetyl group to produce a prodrug with the sequence ac-GKAFRR-L12ADT (SEQ ID NO:41) (FIG. 3). Prior to synthesizing the hK2 TG prodrug, it was essential to determine if hK2 could

still effectively hydrolyze an acetylated peptide in which the Dap in P'1 position is replaced with leucine and where both the nitrotyrosine and lysine-ABZ were absent. Therefore the peptide Y'GKAFRRLGK' (SEQ ID NO:21) was synthesized to analyze the effect of substituting Dap in the P'1 position for leucine. Additionally a peptide ac-GKAFRRLG (SEQ ID NO:~~4322~~) was synthesized to determine effects of acetylation and removal of Y' and K' on hK2 hydrolysis rates. Substitution of Dap-F with Leu-Gly resulted in only a modest decrease in hydrolysis rate (compare FIG. 2, triangles with FIG. 8, diamonds). In addition, BPLC analysis of hydrolysis of the non-fluorescence quenched acGKAFRR-LG (SEQ ID NO:43) peptide demonstrated that this peptide was rapidly hydrolyzed by hK2.

Amended Paragraph [0094] on Page 10

Please replace paragraph [0094] on page 10 with the following replacement paragraph. Amended paragraph [0094] contains a SEQ ID reference for ac-GKAFRR-L12ADT.

[0094] On the basis of these results, ac-GKAFRR-L12ADT (SEQ ID NO:41) was synthesized. This putative hK2 prodrug was incubated with enzymatically active hK2 (4 μg/ml) to determine extent of hydrolysis over time. HPLC analysis of aliquots of the incubation mixture indicated that the hK2 prodrug is rapidly cleaved by hK2 (FIG. 8). MALDI-TOF analysis of the digestion products indicated that cleavage occurred after each arginine residue generating both Arg-Leu-12ADT (RL-12-ADT) and L-12ADT. In 25 min, 50% was hydrolyzed and after 1 hour, more than 80% of the starting prodrug was hydrolyzed. The ratio of the products RL-12ADT:L-12ADT was 1:1.8, as determined by HPLC integration.

Amended Paragraph [0095] on Page 10

Please replace paragraph [0095] on page 10 with the following replacement paragraph. Amended paragraph [0095] contains a SEQ ID reference for ac-GKAFRRLG.

[0095] Not unexpectedly, the time required to reach 50% hydrolysis is slightly longer for the L12ADT prodrug as compared to the peptide ac-GKAFRRLG (SEQ ID NO:43) (FIG. 8, crosses vs. triangles). This difference is most likely is due to substitution of the bulky, hydrophobic 12ADT moiety for the amino acids in the P'2 and P'3 position.

Amended Paragraph [0097] on Page 10, Continuing on Page 11

Please replace paragraph [0097] on page 10 (continuing on page 11) with the following replacement paragraph. Amended paragraph [0097] contains a SEQ ID reference for ac-Y'GKAFRRLGK' and ac-GKAFRR-L12ADT. The sequence ac-Y'GSKFRRLGK' has been assigned SEQ ID NO:18. SEQ ID NO:18 was previously mis-assigned to a sequence defined elsewhere with a different sequence identifier. The mis-assignment of SEQ ID NO:18 was corrected by an amendment presented hereinabove.

[0097] To determine whether merely the acetylation of the N-terminus of the hK2 prodrug contributed to its stability, an acetylated fluorescence-quenched peptide (ac-Y'GKAFRRLGK'; SEQ ID NO:18) was synthesized and compared its plasma stability with that of the non-acetylated peptide (Y'GKAFRRLGK'; SEQ ID NO:21). Surprisingly, hydrolysis of these two peptides was completely identical as judged from the generation of fluorescence in the plate-reader assay. Analytical HPLC indicated the same result. Evidently, acetylation of the N-terminus does not explain the difference in stability between the peptide and the peptide-drug conjugate. The paradoxical stability of the hK2 prodrug (ac-GKAFRR-L12ADT; SEQ ID NO:41) compared to the peptide substrates may also be due to binding of the L12ADT moiety to plasma proteins that make it inaccessible to the plasma protease activity that is responsible for hydrolysis of unconjugated (and unbound) peptides.

Amended Paragraph [0098] on Page 11

Please replace paragraph [0098] on page 11 with the following replacement paragraph. Amended paragraph [0098] contains a SEQ ID reference for ac-GKAFRR-L12ADT.

[0098] Balb-C mice were treated in groups of 3 with a single intravenous injection of increasing doses of the ac-GKAFRR-L12ADT (SEQ ID NO:41) prodrug to establish the dose that killed 100% of mice (i.e. LD_{100}). In these studies the LD_{100} was determined to be 11 μmoles/kg (i.e. 18.2 mg/kg). AU mice, however, tolerated a single intravenous dose of 3.67 μmoles/kg and this dose was then used for further dosing and pharmacokinetic studies. An additional group of mice (n=8) received five consecutive daily intravenous injections with 3.67 μmoles/kg prodrug without any deaths or observable toxicity (i.e. weight loss <15% over baseline).

Amended Paragraph [0099] on Page 11

Please replace paragraph [0099] on page 11 with the following replacement paragraph. Amended paragraph [0099] contains a SEQ ID reference for ac-GKAFRR-L12ADT.

[0099] To determine pharmacokinetic parameters for the hK2-activated thapsigargin prodrug, Balb-C mice (n=3/timepoint) were treated with a single intravenous dose of 3.67 μmoles/kg of the ac-GKAFRR-L12ADT (SEQ ID NO:41) prodrug. At various time points (5, 10, 30 minutes and 1, 1.5, 2, 3, 4, 6, 12, 24 hrs) mice were sacrificed after blood was obtained by cardiac puncture. After precipitating serum proteins with acetonitrile, supernatants were evaluated by LC-MS to determine concentrations of ac-GKAFRR-L12ADT (SEQ ID NO:41), R-L12ADT and L12ADT at each time points. Areas under the curve were converted to concentrations based on a standard curve that was linear for concentrations ranging from 1 nM to 10,000 nM. In this study, the Cmax occurred at 10 minutes post injection and was 36.8 $\pm$ 7.2 μM (FIG. 9). The half-life of the prodrug was 40.7 $\pm$ 1.2 minutes and the area under the curve was 2444.8 $\pm$ 39.1 μmol*min/l (FIG. 9). Both RL12ADT and L12ADT were below the lower limit of detection (i.e. <1 nM) for all time points, FIG. 6. On the basis of these studies, we concluded that the ac-GKAFRR-L12ADT (SEQ ID NO:41) prodrug is highly stable to hydrolysis in the serum in vivo.

Amended Paragraph [0100] on Page 11

Please replace paragraph [0100] on page 11 with the following replacement paragraph. Amended paragraph [0100] contains a corrected SEQ ID reference for the sequence GKAFRRL, as set forth in paragraph [0019]. Amended paragraph [0100] also contains a SEQ ID reference for GKAFRR-L12ADT.

[0100] Due to their short length, the hK2 peptide substrates could potentially be substrates for other trypsin-like proteases. Although a rather specific protease substrate can be defined with 7 amino acids, there is a lack of higher order structural information by which natural protein substrates normally impose high specificity. Cancer progression is often correlated with increased protease activity (Koblinsli, J. E. et al. (2000) Clin. Chim. Acta 291:113-135). These activities could be potentially beneficial since they could broaden the scope of applications for protease activated prodrugs. To test the hypothesis that other tumor-associated proteases could

activate the prodrug, a number of known proteases (table 3), implicated in cancer progression were selected to determine if our lead substrate, GKAFRRL (SEQ ID NO:3022) could be efficiently hydrolyzed by any of these proteases. For this analysis, the trypsin-like serine proteases plasmin and urokinase and cathepsins B and D were selected. Hydrolysis of both the fluorescence quenched peptide substrate and the TG-prodrug was analyzed. No appreciable hydrolysis of either substrate was observed following incubation with Cathepsin D or Cathepsin B. Urokinase showed low activity on the fluorescence quenched peptide substrate, but not on the prodrug (Table 1). Plasmin had a more than 10-fold slower rate of hydrolysis of the peptide substrate than hK2. However, with the prodrug, plasmin had an approximately 6-fold higher hydrolysis rate than hK2. Analysis of the cleavage products demonstrated that with plasmin, proteolysis occurs between the two arginines, generating the less potent cytotoxin Arg-Leu-12 ADT. Plasmin, therefore, could be a valid target for selective activation of the GKAFRR-L12ADT (SEQ ID NO:44) prodrug in other types of cancer where plasmin activation may play an important role.